cultures of human airway smooth muscle cells endogenously expressing $GABA_A$ channels composed of α4, α5 β3 γ1, γ2, δ and θ subunits. We obtained success rates above 95% for transiently or stably transfected HEK cells and frozen "ready to assay" HEK cells expressing $GABA_A$ channels. Tissue-derived immortalized cultures of airway smooth muscle cells exhibited a slightly lower recording success rate of 75% using automated patch, which was much higher than the 5% success rate using manual patch clamp. Primary cells harvested from tissue yielded 30% success on this automated electrophysiology platform. In all cases, both responses to agonist (EC_{50} measurements) and pharmacology of GABA modulators and inhibitors compared well to previously reported manual patch results. The data presented here demonstrates that both the biophysics and pharmacologic characterization of $GABA_A$ channels in a wide variety of cell formats can be performed on this automated patch clamp system. This automated approach exhibited a much higher success rate, lower variability, and higher experimental throughput as compared to manual patch clamp techniques.

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Mapping GABAA Receptor Single Nucleotide Polymorphisms (SNPs) Linked to Epilepsy: Insights into the Receptor Gating and Assembly

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Missense polymorphisms or variants that affect function and/or expression of $GABA_A$ receptors (GABARs) have been associated with idiopathic generalized epilepsies (IGEs) in *GABRA1, GABRA6, GABRB3, GABRG2 and GABRD* genes. Three separate IGE-associated mutations were identified in GABAR subunits (β3(G32R), α6(Q237R) and γ2(K328M)), which represent a large range of GABARs in the nervous system. We sought to investigate the contributions of these mutations into the assembly and function of GABARs. Homology modeling suggested that G32R is located within the N-terminal α-helix β3 subunit domain, and Q237R and K328M are located within the pre-M1 segment of the N-terminal α6 subunit domain and the M2-M3 loop of the N-terminal γ2 subunit domain, respectively. We studied gating properties and surface expression of wild type (wt) α1β3γ2, α6β2γ2, α1β2γ2 and mutant α1β3(G32R)γ2, α6(Q237R)β2γ2, α1β2γ2(K328M) receptors expressed in HEK293T cells. We found that the mutations share common gating defects, but distinctive trafficking defects. Thus, mutant β3(G32R) subunits displayed a mixed profile, causing both gating and trafficking defects of α1β3γ2 receptors, whereas mutant α6(Q237R) and γ2(K328M) subunits caused exclusive channel gating defects of α6β2γ2 and α1β2γ2 receptors. Unexpected, homology modeling predicted that the β3(G32R) mutation affects a salt bridge across the γ2/β3 subunit interface, which underlies an assembly motif reported to be essential for inter-subunit interactions in assembled receptors. In contrast, α6(Q237R) and γ2(K328M) subunit mutations are predicted to interact with residues in Pre-M1 domain, M2-M3 loop and Cys-loop of α6 and γ2 subunits that are critical for desensitization-deactivation coupling of GABARs.

NIH funding NS33300 to RLM.

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Interactions of GABAA Receptors with Steroid-Like Positive and Negative Modulators

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A new model for the gamma-aminobutyric acid class A (GABAa) receptor is presented, based on recent crystal structures of the homologous eukaryotic GluCl channel (3RHW). The models suggest an additional disulfide bridge in the transmembrane domain of both alpha and gamma (but not beta) subunits. Models for the GABAa receptor in complex with steroid or steroid-like modulators such as cholesterol, pregnenolone, and thyroid hormone have also been developed, based on ivermectin sites in PDB:3RHW and mutagenesis studies. Extended molecular dynamics simulations of these models indicate stable binding interactions between the modulator and the receptor, with moderate adjustment of modulator orientation. Hydrogen bonding patterns between modulator and receptor polar groups are shown to be modulator-dependent; these results will be compared to prevailing hypotheses for essential pharmacophores in modulation of GABAa receptors by neurosteroids.

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Optical Control of Neuronal Inhibition with Genetically Engineered Light Inhibited GABAA Receptors (Li-GABARs)

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GABA is the main inhibitory neurotransmitter in the brain, acting primarily at ionotropic $GABA_A$ receptors. The α subunit is critical in determining $GABA_A$ receptor functional properties and pharmacological regulation. There are six α subunit subtypes that are differentially expressed in the nervous system but few selective antagonists are available to dissect the functional role of those subtypes in neurons. We have therefore engineered α subunit specific light inhibited $GABA_A$ receptors (Li-GABARs). We generated α subunits with a cysteine mutation that allows attachment of a photoswitchable tethered ligand (PTL) consisting of a cysteine reactive maleimide group, a photoisomerizable azobenzene core and a variable GABAR ligand. Using cysteine-scanning mutagenesis we identified optimal photoswitch attachment sites near the GABA binding pocket for both α1 and α5 subunits, and tested multiple PTLs to produce maximal light dependent block of GABAR activity while minimally affecting basal receptor properties. We recorded from hippocampal slices expressing Li-GABARs and found evidence that α5, but not α1 containing receptors counteract NMDA receptor dependent synaptic depolarization. This novel interaction could provide a basis for the regulation of synaptic plasticity and memory by α5 receptors. This approach in general provides a strategy to study the role of specific $GABA_A$ receptor subunits and their importance in neuronal function and disease.

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A Molecular Dynamics Study on the Effect of Disulfide Bonds in Cys-Loop Ligand-Gated GABAA Receptors

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GABAA-receptors (GABARs) are chloride ion channels in the 'cys-loop receptor' superfamily that are major inhibitory neuroreceptors. Upon agonist binding, GABARs open and increase intraneuronal chloride ion concentration, hyperpolarize the cell and inhibit neural transmission. Reducing agents will break the disulfide bonds in the cys-loop of the receptor. Indeed, it has been speculated that sulfhydryl compounds modify proteins via thiol/disulfide redox reactions and serve as neuromodulators (1). Reducing reagents were found to decrease the GABA EC50 in the highly homologous GABAC-receptor, while oxidizing reagents increased the EC50 (2). Conversely, conflicting findings suggests the mechanism of action of redox modulation does not alter GABAR agonist-binding affinity (3). However, studies are complicated by the fact that mutagenesis of the cysteines commonly produces failure of subunit assembly (4). Thus, to investigate the effect of disulfide bond breakage on the receptor we generated various GABAR homology models with and without disulfide bonds. These systems were subjected to ligand docking and extensive molecular dynamics simulations to determine if the GABA affinity was modulated. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

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LLNL-ABS-502871

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Allosteric Modulators Targeting Multiple Binding Sites in Bacterial Homolog of GABAA Receptor

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GABAA receptors belong to the family of pentameric ligand-gated ion channels (LGIC), which are involved in fast inhibitory neurotransmission. GABAA receptors are allosterically modulated by benzodiazepines, general anesthetics and alcohols. In this study, we investigate the effects of a range of general anesthetics, alcohols and non-competitive inhibitors on ELIC, which is a recently characterized prokaryote homolog of GABAA receptors. We used two-electrode voltage clamp recordings to identify ELIC modulators from a library of compounds including chlorpromazine, memantine, picrotoxin, etomidate, DMCM and brominated derivatives of alcohols and chloroform. X-ray crystal structures of ELIC in complex with different modulators reveal the molecular architecture for ligand recognition. Consistent with the observation that

allosteric modulators bind at multiple binding sites, we find ligands at different sites in the channel pore, transmembrane domain and ligand-binding domain. In combination with mutagenesis in eukaryote receptors, we probe the importance of homologous residues involved in ligand contacts. Together, our results provide a framework for structure-based design of new allosteric modulators targeting different sites in pentameric ligand-gated ion channels.

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Structural Principles of Serotonin and Granisetron Recognition in a 5-HT3 / Binding Protein Chimera

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The 5-HT3 serotonin receptor belongs to the family of pentameric ligand-gated ion channels (LGIC). A wealth of structural information on molecular recognition of nicotinic ligands has emerged from high-resolution co-crystal structures of acetylcholine binding proteins (AChBPs), which are homologous to the extracellular domain of the nicotinic acetylcholine receptor. Such information is currently missing for other members of the LGIC family, including the 5-HT3 receptor. To overcome this limitation, we used a rational approach to engineer a binding protein with ligand recognition properties similar to the 5-HT3 receptor. Amino acids contributing to binding site loops A-F in AChBP were substituted to their corresponding residues in the 5-HT3 receptor and the effect of these substitutions were characterized with a competitive binding assay using 3H-granisetron. We present crystal structures of a binding protein chimera in complex with agonists and antagonists of the 5-HT3 receptor. Together, this information provides a structural framework for understanding ligand recognition in the 5-HT3 receptor.

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Differential Functional Requirements at the Binding Site Between Serotonin and the Partial Agonist M-Chlorophenylbiguanide in Serotonin 3A Receptors

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The Serotonin 3a Receptor ($5HT3_aR$) is a pentameric ligand gated ion channel found in the central and peripheral nervous systems and implicated in numerous diseases. In previous studies with the endogenous agonist serotonin, we have identified two interactions critical for receptor function: a cation-π interaction at W183 in loop B and a hydrogen bond at E129 in loop A. Here we employ mutant cycle analyses utilizing conventional and unnatural amino acid mutagenesis to investigate how a third residue, D124 of loop A, orients the aforementioned residues for proper receptor function. We also identify differences in receptor binding and the initiation of channel gating between serotonin and the competitive partial agonist *m*-chlorophenylbiguanide (mCPBG) at these and adjacent residues.

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The Molecular Mechanism for the Dual Alcohol Modulation of Cys-Loop Receptors

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Despite high sequence identity, pentameric ligand-gated ion channels exhibit remarkable diversity in function with anionic/cationic channels that are either potentiated or inhibited by allosteric ligands. The recently available structures of bacterial homologs of Cys-loop receptors provide an excellent framework for understanding this allosteric modulation and function, but the modeling can be complex; our earlier simulations of the prokaryotic anionic glycine receptor (GlyR) suggest inter-subunit binding for ethanol (Murail, Biophys J **100**, 1642, 2011), which at first sight appears to be incompatible with the experimental *Gloeobacter violaceus* (GLIC) ligand-gated ion channel structure showing binding intra-subunit.

Here, we present new simulations of GLIC that confirm the occurrence of multiple binding sites by showing intra-subunit binding for ethanol. By experimentally introducing the single-site F238A mutation in GLIC we can turn it into a highly ethanol-sensitive channel (Howard, PNAS **108**, 12149, 2011), similar to GlyR, and simulations of the mutated species confirm the occurrence of multiple binding sites. To critically test the results, we performed extensive docking and free energy calculations to identify alcohol-binding sites and determine their affinity. In the wild-type GLIC, short alcohols preferentially bind intra-subunit, with a very weak binding site inter-subunit. However, with the F238A mutation the inter-subunit site achieves a significantly lower free energy, and even becomes the highest-affinity site in the channel for some alcohols.

These results suggest a new model for pentameric ligand-gated channel potentiation and inhibition, where the intra-subunit cavity would control inhibition, and the inter-subunit cavity potentiation. The possibility of allosteric ligands interacting with both cavities - or even a single molecule stretching from one site to the other - offers an attractive explanation for the complex functional dynamics of ligand-gated ion channels as well as an potential explain for the alcohol cutoff effect.

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Assay Development for Ligand Gated Ion Channel Pharmacology

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Modulators of ligand gated ion channel (LGIC) activity are being actively developed by a number of leading pharmaceutical companies. Electrophysiology assays remain the gold standard for determining functional compound effects on these targets, and pose unique challenges due to the need for accurate temporal control of agonist and compound application.

In this study we present results from complex assays enabled by a novel microfluidic automated patch clamp platform. The assay development process include evaluation of a number of protocols including pre-incubation, co-application, and open channel modulation modalities. The data includes case studies from GABAA, nicotinic and NMDA receptors and trade-offs between the different available measurement modalities developed.

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Agonist Interactions and Selectivity in Inhibitory Cys-Loop Ligand-Gated Ion Channels

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Cys-loop ligand-gated ion channels constitute one of two major superfamilies of receptors mediating rapid chemical synaptic transmission in the central nervous system. Mammalian members of the superfamily include cation selective channels that are receptors for excitatory neurotransmitters, acetylcholine and serotonin, and anion selective channels that are receptors for inhibitory neurotransmitters, GABA and glycine. Structural information from snail acetylcholine binding proteins (AChBP), torpedo acetylcholine receptors provided a clear picture of the acetylcholine-binding site, including the conserved aromatic box which forms cation-pi bonds with bound agonists. From AChBP-based homology models, we proposed (Cromer 2002) that the 3-dimensional position of one of the aromatic box residues is replaced with an acidic residue in inhibitory receptors for GABA (GABAA/CR) and glycine (GlyR). Further that this residue forms a salt-bridge interaction with the cation group of bound agonists and is important for selectivity for primary amine agonists, such as GABA and glycine, over bulkier quaternary amines, such as acetylcholine.

We now present evidence in support of this hypothesis, particularly using the homopentameric Rho1 GABA receptor (GABAC) as a model for the broader family of inhibitory Cys-loop LGICs. We also present evidence for a series of other interactions in the agonist-binding site of inhibitory Cys-loop LGICs that are determinants of selectivity between agonists of different size, such as glycine versus GABA. These results are consistent with our initial hypothesis and provide a more detailed understanding of agonist-receptor interactions in inhibitory Cys-loop ligand-gated ion channels.

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Structure and Function of ELIC Bound with the Antagonist Acetylcholine

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The bacterium Erwinia chrysanthemi ligand gated ion channel (ELIC) is a prototype for Cys-loop receptors, including nicotinic acetylcholine receptors (nAChRs). We co-crystallized ELIC with acetylcholine, an endogenous agonist of nAChRs, and solved the crystal structure to a resolution of 2.9 Å. Acetylcholine binds to the orthosteric agonist site for Cys-loop receptors. Upon acetylcholine binding, loop C exhibits a substantial contraction with a profound reduction in flexibility. Conformational changes in nearby regions of the ligand-binding domain are also observed. However, no substantial reorganization in the pore